CLAIMS

1. A method for identifying and/or cloning nucleic acid regions representative of qualitative genetic differences occurring between two biological samples, comprising hybridizing RNAs or double stranded cDNAs derived from a first biological sample with cDNAs derived from a second biological sample.

2. A method according to claim 1, wherein said method comprises :

(a) hybridizing RNAs derived from the first sample (test condition) with cDNAs derived from the second sample (reference condition);

(b) hybridizing RNAs derived from the second sample (reference condition) with cDNAs derived from the first sample (test condition) ; and

(c) identifying and/or cloning, from the hybrids formed in steps (a) and (b), nucleic acids corresponding to qualitative genetic differences.

3. A method according to claim 1 or 2, wherein the hybridizations are performed between RNAs and single stranded cDNAs and wherein it comprises identifying and/or cloning unpaired RNA regions.

4. A method according to claim 1 or 2, wherein the hybridizations are performed between RNAs and double stranded cDNAs and wherein it comprises identifying and/or cloning paired DNA regions.

5. A method according to claim 1, wherein it comprises hybridizing double stranded cDNAs derived from a first biological sample with single stranded cDNAs derived from a second biological sample.

6. A method according to any one of claims 1 to 5, wherein the biological sample consists of cells, a tissue, an organ or a biopsy sample.

7. A method according to any one of claims 1 to 6 for identifying and/or cloning differential alternative forms of splicing occurring between tumoral and non tumoral cells.

8. A method according to any one of claims 1 to 6 for identifying and/or cloning differential alternative forms of splicing occurring between cells treated by a test compound and non treated cells.

9. A method according to any one of claims 1 to 6 for identifying and/or cloning differential alternative forms of splicing occurring between cells undergoing apoptosis and non apoptotic cells.

10. A method according to any one of claims 1 to 9, wherein hybridization is performed in a liquid phase.

11. A method for identifying and/or cloning differentially spliced nucleic acid regions occurring between two physiological conditions A and B, comprising :

(a) generating heteroduplex structures in a liquid phase between the messenger RNAs derived from condition A and the cDNAs derived from condition B on the one hand;

(b) generating heteroduplex structures in a liquid phase between the messenger RNAs derived from condition B and the cDNAs derived from condition A on the other hand ; and

(c) identifying and/or cloning unpaired RNA regions within the heteroduplex structures obtained in steps (a) and (b).

12. A method for identifying and/or cloning differentially spliced nucleic acid regions occurring between two physiological conditions A and B, comprising :

(a) generating heteroduplex structures between the messenger RNAs derived from condition A and the cDNAs derived from condition B on the one hand, the RNAs or cDNAs being fixed to a support material ;

(b) generating heteroduplex structures between the messenger RNAs derived from condition B and the cDNAs derived from condition A on the other hand, the RNAs or cDNAs being fixed to a support material; and

(c) identifying and/or cloning unpaired RNA regions within the heteroduplex structures obtained in steps (a) and (b).

13. A composition comprising nucleic acids identified and/or cloned

according to the methods of claims 1 to 12.

14. A nucleic acid composition, wherein said composition essentially comprises nucleic acids representative of qualitative genetic differences, notably alternative forms of splicing distinctive of two physiological conditions of a cell or tissue.

15. A composition according to claims 13 or 14, wherein the nucleic acids are cloned into vectors.

16. A nucleic acid library comprising nucleic acids specific to qualitative genetic alterations, notably alternative forms of splicing which are distinctive of two physiological conditions of a cell or tissue.

17. A library according to claim 16, wherein said library is a library restricted to alternative forms of splicing characteristic of mature RNAs.

18. A library according to claim 16, wherein said library is a complex library of alternative forms of splicing characteristic of transcripts.

19. A library according to claim 16, wherein said library is an autologous library characteristic of alternative forms of splicing occurring between mature and premessenger RNAs of a given physiological condition.

20. A nucleic acid library comprising oligonucleotides or PCR fragments specific to alternative forms of splicing which are distinctive of two physiological conditions.

21. A microorganism library comprising microorganisms transformed by nucleic acids specific to alternative forms of splicing which are distinctive of two physiological conditions of a cell or tissue.

22. A library according to claims 16 to 21, wherein said library is deposited on a support material.

23. A kit comprising a support material upon which is deposited a library according to any one of claims 16 to 21.

24. A kit according to claim 23, wherein said kit comprises two libraries according to any one of claims 16 to 21, deposited on a common support or on two individual support materials.

25. A kit according to claims 23 or 24, wherein the support material consists of a filter, membrane or chip.

26. The use of a composition according to any one of claims 13 to 15 or of a library according to claims 16 to 21 for identifying active molecules.

27. The use of a composition according to any one of claims 13 to 15 or of a library according to claims 16 to 21 for identifying proteins or protein domains which are altered in a pathology.

28. The use of a composition according to any one of claims 13 to 15 or of a library according to claims 16 to 21 for identifying antigenic domains specific to proteins involved in a pathology.

29. A method for identifying and/or producing proteins or protein domains involved in a pathology comprising :

(a) hybridizing the messenger RNAs of a pathological sample with the cDNAs derived from a healthy sample, or vice versa, or both in parallel,

(b) identifying, within the hybrids formed, the regions corresponding to qualitative differences specific to the pathological state as compared to the healthy state,

(c) identifying and/or producing the protein or protein domain corresponding to a region identified in step (b).

30. A method for identifying and/or cloning tumor suppressor genes or splicings within tumor suppressor genes, comprising :

(a) hybridizing the messenger RNAs of a tumor sample with the cDNAs of a healthy sample, or vice versa, or both in parallel,

(b) identifying, within the hybrids formed, the regions corresponding to qualitative differences specific to the tumor sample as compared to the healthy sample,

(c) identifying and/or producing a protein or protein domain corresponding to a region identified in step (b).

31. A composition comprising a compound capable of interfering with the products of alternative splicings identified according to the methods of claims 1 to 12.

32. A protein identified using the method of claim 29.

33. The use of a library according to claims 16 to 21 or of a kit according to claims 23 to 25 to evaluate the toxicity of a compound.

34. A method for identifying and/or cloning nucleic acids specific to a toxic state of a given biological sample comprising preparing qualitative differential libraries between the RNAs and the cDNAs from the sample after treatment or no treatment by a toxic test compound, and screening for toxicity markers specific of the characteristics of the sample post-treatment.

35. A method for determining or assessing the toxicity of a test compound to a given biological sample comprising hybridizing :

- differential libraries between the cDNAs and the RNAs of said biological sample in healthy condition and at one or different stages of toxicity as a result of treating said sample with a reference toxic compound, with,
- a nucleic acid preparation of the biological sample treated by said test compound, and
- assessing the toxicity of the test compound by examining the extent of hybridization with the different libraries.

36. A method according to claim 35, wherein the biological sample is a

culture of hepatocytes, renal epithelial cells or endothelial cells, either subjected or not to treatment by a toxic agent, preferably ethanol.

37. A method according to claim 35, wherein the biological sample is a skin culture either subjected or not to treatment by toxic agents or irritants.

38. The use of a library according to claims 16 to 21 or of a kit according to claims 23 to 25 to evaluate the potency of a compound.

39. A method for determining or assessing the therapeutic efficacy of a test compound with respect to a given biological sample comprising hybridizing :

- differential libraries between the cDNAs and the RNAs of said biological sample in healthy and pathological conditions, with,
- a nucleic acid preparation of the biological sample treated by said test compound, and
- assessing the therapeutic potential of the test compound by examining the extent of hybridization with the different libraries.

40. The use of a library according to claims 16 to 21 or of a kit according to claims 23 to 25 to evaluate the responsiveness of a pathological sample to a compound.

41. A method for determining or assessing the responsiveness of a patient to a test compound or treatment comprising hybridizing :

- differential libraries between the cDNAs and the RNAs of a biological sample responsive to said compound/treatment and of a biological sample unresponsive or poorly responsive to said compound/treatment, with,
- a nucleic acid preparation of a pathological biological sample of the patient, and
- assessing the responsiveness of the patient by examining the extent of hybridization with the different libraries.

42. A method according to claim 41 for determining or assessing the response of a patient to an anti-tumoral compound or treatment.

43. A method according to claim 42 for determining or assessing the response of a patient to an antitumoral treatment through wild type p53 gene transfer.

44. A nucleic acid identified by the method according to claims 1 to 12.

45. The use of a nucleic acid according to claim 44 for detecting genetic defects in a sample.

46. The use of a compound according to claim 28 for detecting a genetic defect in a sample.

47. An antibody directed against a protein or protein domain as defined in claim 27 or 28.

48. The ΔSHC protein with the sequence SEQ ID NO: 9.

49. A nucleic acid probe, oligonucleotide or antibody for identifying the ΔSHC protein according to claim 48 or its nucleic acid, and/or a modification of the SHC/ΔSHC ratio in a biological sample.

50. A screening method, wherein said method is based on blocking the spliced domain in the SHC protein or inhibiting the functions acquired by the spliced protein ΔSHC.

51. A vector comprising a sequence coding for the ΔSHC protein according to claim 48.

52. A method according to claims 3, 4 or 5, wherein cloning nucleic acids comprises reverse transcription and/or amplification by means of random or semi-random primers, particularly primers with sequence SEQ ID NO: 3 in which N indicates that each of the four bases may be present randomly at the indicated position, W, X and Y each designate a defined base, and Z designates either a

defined base, or a 3'-OH group.

53. An oligonucleotide comprising, in the 5' ---> 3' orientation :

- a stabilizing region comprising 8 to 24 defined nucleotides,
- a random region comprising 3 to 8 nucleotides, and
- a minimal priming region comprising 2 to 4 defined nucleotides.

54. An oligonucleotide with sequence SEQ ID NO: 3 in which :

- N indicates that each of the four bases may be present randomly at the indicated position;
- W, X and Y each designate a defined base,
- Z designates either a defined base, or a 3'-OH group.

55. A genomic DNA library, wherein said library consists of genomic DNA fragments whose size is less than or equal to approximately 1 kb.

56. A method for detecting or monitoring the toxicity and/or therapeutic potential of a compound, based on detecting the splicing forms and/or profiles induced by said compound on a biological sample.

57. The use as a source of pharmacogenomic markers of (i) interindividual variability of isoforms generated by alternative splicing (spliceosome analysis) or (ii) splicing alterations induced by treatments.